COBAS AmpliScreen HCV Test, v2.0

Summary of Basis for Approval

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Trade Name	COBAS AmpliScreen HCV Test, v2.0
Proper Name (Licensed Name)	Hepatitis C Virus / Polymerase Chain Reaction / Blood Cell Derived [COBAS AmpliScreen™]
Applicant / Manufacturer	Roche Molecular Systems, Inc. 4300 Hacienda Drive Pleasanton, CA 94588 FDA Registration No: 2243471
Biological License Application (BLA) Reference Number(s)	STN 125045/0
Report Date	December 3, 2002

I. INTENDED USE

The COBAS AmpliScreen Hepatitis C Virus (HCV) Test, version 2.0 (v2.0) is a qualitative *in vitro* test for the direct detection of Hepatitis C Virus RNA in human plasma from donations of whole blood and blood components for transfusion.

The test is intended for use in screening individual donor samples of human plasma, or pools of human plasma comprised of equal aliquots of not more than 24 individual donations. The test is intended to be used for detecting HCV RNA in conjunction with licensed tests for detecting antibodies to HCV.

This assay is not intended for use as an aid in diagnosis.

II. BRIEF DESCRIPTION OF DEVICE AND PRINCIPLES

A. Summary and Explanation of the Test

The COBAS AmpliScreen HCV Test, v2.0, uses a generic sample preparation technique in a mini-pool testing format along with automated amplification and detection using PCR on the COBAS AMPLICOR Analyzer for the detection of HCV RNA in blood donations. The assay incorporates an Internal Control for monitoring assay performance in each individual test as well as AmpErase® to reduce potential contamination by previously amplified material (amplicon).

The COBAS AmpliScreen HCV Test, v2.0 is based on five major processes:

Sample processing, reverse transcription of target RNA to generate complementary DNA (cDNA), PCR amplification of target cDNA using HCV-specific complementary primers, hybridization of the amplified products to oligonucleotide probes specific to the target(s), and detection of the probe-bound amplified products by colorimetric determination.

Two specimen preparation procedures are used with the AmpliScreen HCV Test, v2.0 as follows:

- Multiprep Specimen Processing procedure for preparation of mini-pool specimens
- Standard Specimen Processing for preparation of individual donor samples

In the Standard Specimen Processing procedure, HCV RNA is isolated directly from plasma by lysis of the virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol. In the Multiprep Specimen Processing procedure, HCV viral particles are first pelleted from the plasma sample by high speed centrifugation, followed by lysis of the pelleted virus with a chaotropic agent and precipitation of the RNA with alcohol.

The Multiprep Internal Control, containing the HCV Internal Control is introduced into each sample and serves as an extraction and amplification control for each processed specimen and control. The HCV Internal Control is an RNA transcript with primer binding regions identical to those of the HCV target sequence, a randomized internal sequence of similar length and base composition as the HCV target sequence, and a unique probe binding region that differentiates the HCV Internal Control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the HCV Internal Control and the HCV target RNA.

The reverse transcription and amplification reactions are performed with the thermostable recombinant enzyme Thermus thermophilus DNA Polymerase (r*Tth* pol). r*Tth* pol has both reverse transcriptase and DNA polymerase activity.[29] This allows both reverse transcription and PCR amplification to occur in the same reaction mixture. Reverse transcription using r*Tth* pol produces a cDNA copy of the HCV target and the HCV Internal Control RNA.

Following reverse transcription a second DNA strand is produced from the cDNA copy, thereby yielding a double-stranded DNA copy of the target region of the HCV and HCV Internal Control RNA. The reaction mixture is heated again to separate the resulting double-stranded DNA, and extends the annealed primers along the target templates to produce a double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automatically repeats this process for a designated number of cycles, each cycle effectively doubling the amount of amplicon DNA.

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds denaturation solution to chemically denature the HCV amplicon and the HCV Internal Control amplicon to form single-stranded DNA. A suspension of magnetic particles coated with an oligonucleotide probe specific for HCV amplicon or HCV Internal Control amplicon is added. The biotin-labeled HCV target and HCV Internal Control amplicon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles.

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles to remove unbound material, and then adds Avidin-Horseradish Peroxidase Conjugate. The Avidin-Horseradish Peroxidase Conjugate binds to the hybridized biotin-labeled amplicon. The COBAS AMPLICOR Analyzer adds a substrate solution containing hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB). In the presence of hydrogen peroxide, the particle-bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex. The absorbance is measured by the COBAS AMPLICOR Analyzer at a wavelength of 660 nm.

B. Description of Kit and Component Formulations

The COBAS AmpliScreen Multiprep Specimen Preparation and Control kit and the COBAS AMPLICOR Wash Buffer kit are provided as stand alone kits to be used in conjunction with the COBAS AmpliScreen HCV Test, v2.0.

COBAS AmpliScreen HCV Test, version 2.0 — 96 Tests

Amplification Reagents

HCV MMX v2.0 (HCV Master Mix, version 2.0) 8 x 0.7 mL

Bicine buffered, DMSO, glycerol, *rTth* DNA Polymerase, potassium acetate, primers, dNTPs, AmpErase and sodium azide as a preservative

HCV Mn^{2+} v2.0 (HCV Manganese Solution, version 2.0) 8 x 0.1 mL

Manganese solution with acetic acid, indicator dye and sodium azide as a preservative

COBAS AmpliScreen HCV Detection Reagents, version 2.0

DN4 (Denaturation Solution) 1 x 100 Tests

EDTA

Thymol blue

Sodium hydroxide

CH PS1 v2.0 (HCV Probe Suspension 1, version 2.0) 1 x 100 Tests

MES buffer solution containing capture oligonucleotides and magnetic microparticles with sodium azide as a preservative

CH4 v2.0 (HCV Probe Suspension 2, version 2.0) 1 x 100 Tests

Sodium phosphate buffer

Sodium thiocyanate

Solubilizer

Sodium thiocyanate

CI PS1 (IC Probe Suspension 1) 1 x 100 Tests

MES buffer solution containing magnetic microparticles with capture olgionucleotides and sodium azide as a preservative

CI4 (IC Probe Suspension 2) 1 x 100 Tests

Sodium phosphate buffer containing sodium thiocyanate

CN4 (Avidin-Horseradish Peroxidase Conjugate) 2 x 100 Tests

Tris-HCl buffer solution containing Avidin-horseradish peroxidase conjugate, bovine serum albumin, Emulsit 25 and phenol with ProClin 150 as a preservative

SB3 (Substrate A) 10 x 75 Tests

Citrate solution containing hydrogen peroxide with ProClin 150 as a preservative

SB (Substrate B) 10 x 75 Test

3,3',5,5'-Tetramethylbenzidine (TMB)

Dimethylformamide (DMF)

Dimethylformamide (DMF)

COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit 96 Tests

MP (+) C (Multiprep Positive Control) 8 x 0.1 mL

Tris-HCl buffered solution containing noninfectious RNA transcripts for HCV and HIV-1 and non-infectious HBV DNA plasmid with EDTA and sodium azide as a preservative.

MP LYS (Multiprep Lysis Reagent) 8 x 9.0 mL

Tris-HCl buffered solution with Dithiothreitol, Glycogen and Guanidine thiocyanate

MP DIL (Multiprep Specimen Diluent) 8 x 4.8 mL

Tris-HCl buffered solution with EDTA and sodium azide as a preservative

MP IC (Multiprep Internal Control) 8 x 0.1 mL

Tris-HCl buffered solution with non-infectious internal control RNA transcripts for HCV and HIV-1 and DNA plasmid for HBV, Poly rA RNA, EDTA, indicator dye and sodium azide as a preservative.

MP (–) C (Multiprep (–) Control) 8 x 0.1 mL

Poly rA RNA, EDTA and sodium azide as a preservative

NHP (Negative Plasma (Human)) 16 x 1.6 mL

Human plasma, non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV, HIV-1 p24 antigen and HBsAg, with ProClin® 300 as a preservative.

COBAS AMPLICOR Wash Buffer 500 Tests

WB (10X-Wash Concentrate) 2 x 250 Tests

Phosphate buffer solution containing detergent with ProClin 300 as a preservative

III. MANUFACTURING AND CONTROLS

A. Description of Manufacturing Facilities

The COBAS AmpliScreen HCV Test, v2.0 is manufactured by Roche Molecular Systems, Inc. (RMS) and prepared under U.S. License 1636. The corporate headquarters is located at 4300 Hacienda Drive, Pleasanton, California. The primary RMS manufacturing facility is located at 11 Franklin Avenue, Belleville, New Jersey. One component of the COBAS AmpliScreen HCV Test, v2.0 (the COBAS AmpliScreen Multiprep Internal Control) is produced in manufacturing laboratories in the RMS facility located at 1080 US Highway 202, Branchburg Township, Somerville, New Jersey (referred to as the Branchburg Facility). The Multiprep Positive Control is one of the Positive Controls manufactured for RMS by Lampire Biological Laboratories, Inc., located at 5185 Applebutter Road, Pipersville, Pennsylvania. The buffers used in the manufacturing of the Positive Controls are supplied by the RMS Belleville facility and RMS Alameda facility (1145 Atlantic Avenue, Alameda, California). Final product is stored and distributed from the RMS Totowa warehouse located at 701 Union Boulevard, Totowa, New Jersey. Finished, approved product is distributed in the United States from the Roche Diagnostic distribution center located in Indianapolis, Indiana.

The DNA oligonucleotide primers (KY80 and KY78) and probes (SK535 and KY150) used in the COBAS AmpliScreen Test, version 2.0 are manufactured synthetically on a DNA synthesizer and purified by HPLC at RMS.

The *Thermus thermopilus* and Uracil-N-Gylcosylase (rUNG) Enzymes used in the Test are manufactured at RMS. Both enzymes are grown in *E. coli* and purified by first chemically disrupting the cells and purifying the enzyme by HPLC.

The RNA Multiprep Positive and Internal Controls include purified RNA transcripts and linearized DNA fragments derived from plasmids grown in *E. coli*. For the HIV and HCV RNA Multiprep Positive (pSYC35 HIV and pHCVIIA HCV control) and Internal Controls (pSDL150 HIV and pSYC52 HCV Control), and HBV Plasmid Multiprep Positive (pCABN positive) and Internal Control DNA (pTMN1 HBV control) is purified

from the *E. Coli* host, by first disrupting the cells, then purifying the plasmid by extraction, gradient centrifugation and alcohol precipitation. RNA transcript is prepared by incubating the purified plasmid DNA with RNA Polymerase and extracting the newly transcribed RNA by precipitating the RNA with alcohol. The RNA is further purified by column chromatography.

The Linearized plasmid DNA is prepared by cutting the purified plasmid DNA with restriction digest enzymes followed by extraction, and alcohol precipitation.

The Negative Control is prepared from Human Plasma pools, which are tested and found to be negative for anti-HCV, HBV and HIV.

The raw materials used in this product are subjected to appropriate quality control evaluations before they are accepted for use in manufacturing. Acceptance criteria and performance specifications have been established for all test kit components. Components are assembled into test kits, each lot of which is subjected to a final performance test.

Each COBAS AmpliScreen HCV Test, version 2.0 kit lot is tested with in-house panels of samples with varying HCV copy numbers/mL, as well as the CBER HCV Reference Panel, and must meet the performance requirements of both panels.

B. Stability Program

Components of the COBAS AmpliScreen HCV Test, v2.0 are entered into the stability program in order to define the recommended storage conditions and to establish the expiration dating period (i.e., shelf life) for each component. The expiration date of the complete test kit is defined on a lot-by-lot basis as the expiration date of the component lot with the shortest expiration date. In addition, components held at or beyond shelf life were assembled into "virtual test kits" and functional testing was performed.

The results of the stability studies completed to date support the shelf-life claims summarized in the table below.

COBAS AmpliScreen HCV Test, v2.0
Quality Control Component Shelf Life, Storage Temperature and Stability Tests

Component	Code	Proposed Shelf Life (months)	Storage Temperature	Real Time Stability Tests
AmpliScreen Multiprep Lysis Reagent	58004067	15	2-8°C	Appearance, Color, pH, Assay (dithiothreitol)
AmpliScreen Multiprep Internal Control	58004065	15	2-8°C	Appearance, Color, Poisson Analysis
AmpliScreen Multiprep Specimen Diluent	58004066	21	2-8°C	Appearance, Color, Performance Test
AmpliScreen HCV Master Mix, v2.0	58004073	15	2-8°C	Appearance, Color, Performance Tests
AmpliScreen HCV Manganese Solution, v2.0	58004072	24	2-8°C	Appearance, Color, Assay (Manganese)
AmpliScreen Multiprep Positive Control	58004251	12	2-8°C	Appearance, Color, Poisson Analysis (HIV, HCV, HBV)
AmpliScreen Multiprep Negative Control	58004064	24	2-8°C	Appearance, Color, Performance Test
Negative Plasma (Human)	58001344	18	2-8°C	Appearance, Color, Performance Tests
COBAS AMPLICOR Denaturation Solution	58001319	24	2-25°C	Appearance, Color, pH
COBAS AmpliScreen HCV Probe Suspension 1, v2.0	58004323	18	2-8°C	Appearance, Color, Performance Test
COBAS AmpliScreen HCV Probe Suspension 2, v2.0	58004324	24	2-8°C	Appearance, Color, pH, Buffering Capacity, Assay (sodium thiocyanate)
COBAS AmpliScreen IC Probe Suspension 1	58004325	24	2-8°C	Appearance, Color, Performance Test
COBAS AmpliScreen IC Probe Suspension 2	58004326	24	2-8°C	Appearance, Color, pH, Buffering Capacity, Assay (sodium thiocyanate)
COBAS AMPLICOR Avidin- HRP Conjugate	58001301	21	2-8°C	Appearance, Color, pH, Performance Test
COBAS AMPLICOR Substrate A	58001318	24	2-8°C	Appearance, Color, pH, Assay (hydrogen peroxide)
COBAS AMPLICOR Substrate B	58001333	24	2-8°C	Appearance, Color, Assay (TMB)
COBAS AMPLICOR 10XWash Concentrate	58001332	24	2-25°C	Appearance, Color, pH, Working Reagent pH, Assay (sodium chloride)

C. Methods of Validation

All test kit components are monitored by in-process testing. Product purity and potency are assured through the evaluation of the product appearance, chemical testing, and performance testing. Product performance is assessed through quality release evaluations of the final test kit against an in-house panel containing negative control specimens and specimens that are known to be positive for HCV virus. A CBER HCV Panel is also tested in this evaluation and the test kit must meet all performance requirements. The AmpliScreen HCV Test, version 2.0 meets the FDA release requirements.

D Labeling

The product labeling, including immediate container labels, box or package labels, and package insert have been reviewed for compliance with 21 CFR§610.60, 610.61, 610.62 and 809.10 and were found acceptable. The package insert clearly states the intended use: as a qualitative *in vitro* test for the direct detection of Hepatitis C Virus RNA in human plasma from donations of whole blood and blood components for transfusion. The test is intended for use in screening of individual donor samples of human plasma or pools of human plasma comprised of equal aliquots of not more than 24 individual donations. The test is intended to be used for detecting HCV RNA in conjunction with licensed tests for detecting antibodies to HCV. This assay is not intended for use as an aid in diagnosis. The product tradename, COBAS AmpliScreen HCV Test, version 2.0 is not known to conflict with any other biologic or device tradename.

E. Establishment Inspection

A Pre-license Inspection of the manufacturing facilities where the COBAS AmpliScreen product lines are manufactured, tested, stored and shipped was conducted from July 8 through July 19, 2002.

F. Environmental Impact Analysis, Claims for a Categorical Exclusion

Roche Molecular Systems, Inc. claimed a Categorical Exclusion from the submission of an Environmental Impact Statement with the COBAS AmpliScreen HCV Test, version 2.0, Biologics License Application. This claim for a Categorical Exclusion was made pursuant to 21 CFR 25.24(e)(4). The manufacture of the COBAS AmpliScreen HCV Test, version 2.0, is performed under controlled conditions and in compliance with the appropriate federal, state, and local environmental regulations. The disposal of waste from the use of this product is performed in compliance with appropriate federal, state, and local environmental regulations. Based on the materials, concentration, volumes used in this product, the method(s) of product disposal, it is unlikely that the release of any of the substances of this product at the expected level of exposure will be harmful to the environment or toxic to organisms in the environment.

IV. PERFORMANCE CHARACTERISTICS

A. Pre-clinical Studies Summary

Pre-clinical performance studies include the determination of the following:

1. Assay Cutoff
2. Analytical Sensitivity
    a. Determination of Limit of Detection (LOD), Using the WHO HCV International Standard, 96/790
    b. Analytical Sensitivity – CBER HCV Panel
    c. Dilutional Sensitivity with Clinical Samples
    d. Sensitivity — Genotype Detectability
    e. Sensitivity with Seroconversion Panels
3. Analytical Specificity
    a. Analytical Specificity: Interfering Microorganism
    b. Analytical Specificity: Specificity with Non-HCV Hepatitis Samples
4. Effect of Interfering Substances
    a. Endogenous Interfering Substances
    b. Exogenous Interfering Substances
5. Uracil-N-glycosylase (UNG) Performance
6. Reproducibility Studies

B. Clinical Trials Summary

Clinical studies include the determination of the following:

1. Pool Reactivity in Volunteer Blood Donors

2. Single Donation Testing Performance

3. Sensitivity in a Chronic HCV Population

4. Detection of Window Period Cases

5. Sensitivity in a High Risk Population

A. Pre-clinical Studies Summary

1. Assay Cutoff

Study Description. The cutoff value was determined by testing seronegative plasma specimens drawn from 502 blood donors, and 161 HCV seropositive specimens drawn from plasma donors. In addition, HCV RNA-positive samples from seroconversion panels were included (38 were processed using the Multiprep Sample Processing Procedure, and 66 were processed using the Standard Sample Processing Procedure). The complete description of all samples included in the determination of the cutoff value is provided in the following table.

Table 1: Determination of the Cutoff Data Summary

Sample Description	n	A_{660} Values	
		Multiprep Sample Preparation*	**Standard Sample Preparation**
Seronegative plasma (EDTA)	502	Range: 0.002 – 0.015 Mean: 0.007 Standard deviation: 0.002.	Range: 0.002 – 0.136 Mean: 0.007 Standard deviation: 0.006.
Seropositive plasma (EDTA)	107	Range: 3.312 – 4.000 Mean: 3.672 Standard deviation: 0.216..	Not Tested
Chronic HCV, Seropositive plasma, (EDTA)	54	Range: 2.14 – 4.0 Mean: 3.255 Standard deviation: 0.358.	Range: 2.932 – 4.0 Mean: 3.509 Standard deviation: 0.238
Seropositive Samples (Seroconversion Panel) Set 1	38	Range: 2.354 – 4.00 Mean: 3.770 Standard deviation: 0.316	Not Tested
Seropositive Samples (Seroconversion Panel) Set 2	66	Not Tested	Range: 1.617 – 4.00 Mean: 3.85 Standard deviation: 0.369.

**Each sample was diluted 1:24 with Negative Human Plasma prior to testing.*

Results

The data show a bimodal distribution of the A_{660} values for true positive and true negative specimens for both the Multiprep and Standard procedures. This is expected for amplified nucleic acid tests where the goal of the procedure is to achieve large absorbance signals in the presence of low target levels. A cut-off value of =0.20 A_{660} was determined appropriate for the COBAS AmpliScreen HCV Test, v2.0 for both the Multiprep and Standard Sample Processing Procedures. Based on study results, a cutoff of =0.20 A_{660} would provide optimal clinical performance for either specimen processing procedure. The following table summarizes the test result validity criteria for Primary Pools, Secondary Pools, and Individual Donor Samples.

Table 2: Cutoff Summary and Test Result Validity Criteria

HCV Result		IC Result		Interpretation
A_{660}	Comment	A_{660}	Comment	
< 0.2	NEGATIVE	≥ 0.2	VALID	Specimen is negative for HCV RNA.
< 0.2	NEGATIVE	< 0.2	INVALID	Invalid result. Repeat entire test procedure for invalid specimen.
≥ 0.2	POSITIVE	ANY	VALID	Specimen is positive for HCV RNA.

2. Analytical Sensitivity

a. Determination of Limit of Detection (LOD) Using the WHO HCV International Standard, 96/790

The analytical sensitivity of the COBAS AmpliScreen HCV Test, v2.0 was determined using the WHO HCV International Standard (96/790). The WHO HCV International Standard was serially diluted in HCV-negative plasma to final concentrations of 200, 100, 50, 25, 15, and 10 IU/mL. Each dilution was tested with two lots of the COBAS AmpliScreen HCV Test, v2.0 using both the Multiprep and Standard Specimen Processing Procedures.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% LOD of 28.8 IU/mL, with lower and upper 95% confidence limits of 20.5 IU/mL and 85.8 IU/mL, respectively.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 41.9 IU/mL, with lower and upper 95% confidence limits of 28.0 IU/mL and 111.8 IU/mL, respectively.

Tables 3 and 4 summarize the overall results for the Multiprep and Standard Specimen Processing Procedures, respectively.

Table 3: Serial Dilution Testing Summary for Multiprep Method Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-sided)
200	132	132	100.00%	97.76%
100	132	132	100.00%	97.76%
50	130	132	98.48%	95.31%
25	128	132	96.97%	93.20%
15	95	132	71.97%	64.83%
10	92	132	69.70%	62.45%

Table 4: Serial Dilution Testing Summary for Standard Method Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-sided)
200	131	131	100.00%	97.74%
100	129	132	97.73%	94.23%
50	132	132	100.00%	97.76%
25	115	132	87.12%	81.31%
15	93	131	70.99%	63.77%
10	84	132	63.64%	56.19%

b. Analytical Sensitivity – CBER HCV Panel

The FDA CBER HCV Panel Members # 1-10 were processed using the Multiprep and Standard Sample Processing Procedures. Both specimen processing methods detected HCV RNA at 50 copies/mL. The Multiprep Sample Processing Procedure detected 100% of all positive members ranging from 10 - 100,000 copies/mL. The Standard Sample Processing Procedure detected 100% of all positive members ranging from 50 to 100,000 copies/mL. Both negative members of the panel were negative by both methods. The data are shown in Table 5.

Table 5: CBER HCV RNA Panel Results

CBER HCV RNA Panel (Copies/mL)	CBER HCV Panel Member Test Results (Percent Positive)									
	1 (1000)	2 (Neg)	3 (100,000)	4 (10,000)	5 (Neg)	6 (500)	7 (200)	8 (50)	9 (10)	10 (5)
Multiprep Method	100%	0%	100%	100%	0%	100%	100%	100%	100%	67%
Standard Prep Method	100%	0%	100%	100%	0%	100%	100%	100%	67%	0%

c. Dilutional Sensitivity with Clinical Samples

The analytical sensitivity of the COBAS AmpliScreen HCV Test, v2.0 was determined by testing 10 HCV seropositive clinical specimens. The titer of each specimen was quantitated with a commercially available assay using a secondary standard calibrated against the WHO International Standard. These specimens were diluted in normal human plasma to 150, 50, 16.7 and 5.6 HCV RNA IU/mL for the Multiprep Specimen Processing Procedure and 300, 100, 33.3 and 11.1 IU/mL for the Standard Specimen Processing Procedure. The COBAS AmpliScreen HCV Test, v2.0 detected 16.7 HCV RNA IU/mL at a frequency greater than 90% with a lower 95% confidence limit of 86.4% using the Multiprep Specimen Processing Procedure. The assay detected 33.3 HCV RNA IU/mL at a frequency greater than 84% with a lower 95% confidence limit of 79.7% using the Standard Specimen Processing Procedure. The data are presented in Tables 6 and 7.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% Limit of Detection (LOD) of 21.0 IU/mL, with lower and upper 95% confidence limits of 17.1 IU/mL and 27.8 IU/mL, respectively. The LOD of 21.0 IU/mL corresponds to approximately 57 copies/mL.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 54.1 IU/mL, with lower and upper 95% confidence limits of 44.1 IU/mL and 71.7 IU/mL, respectively. The LOD of 54.1 IU/mL corresponds to approximately 146 copies/mL.

Table 6: Multiprep Procedure Testing Summary for All Clinical Samples Combined Input Values with 95% One-tailed Lower Confidence Limit

Multiprep Sample Processing Procedure				
HCV RNA Concentration (IU/mL)	**Number of Positives**	**Number of Individual Trials**	**% Positive**	**95% Lower Confidence Limit – One-Tailed**
150	219	219	100.0%	98.6%
50	220	220	100.0%	98.6%
16.7	197	218	90.3%	86.4%
5.6	30	44	68.1%	54.8%

Table 7: Standard Procedure Testing Summary for All Clinical Samples Combined Input Values with 95% One-tailed Lower Confidence Limit

Standard Sample Processing Procedure				
HCV RNA Concentration (IU/mL)	**Number of Positives**	**Number of Individual Trials**	**% Positive**	**95% Lower Confidence Limit – One-Tailed**
300	220	220	100.0%	98.6%
100	220	220	100.0%	98.6%
33.3	183	217	84.3%	79.7%
11.1	54	87	62.1%	52.7%

d. Sensitivity — Genotype Detectability

Twenty individual plasma specimens representing Genotypes 1 and 4, sixteen plasma specimens of Genotype 2, nineteen plasma specimens of Genotype 3, and two plasma specimens each of Genotypes 5 and 6 were tested. With the exception of one sample (Genotype 2a/2c), which was below the limit of quantitation by a quantitative assay, each specimen was diluted to approximately 200 IU/mL of HCV RNA in pooled negative human plasma. Diluted samples were processed using both the Multiprep and Standard Sample Processing Procedures. The COBAS AmpliScreen HCV Test, v2.0 detected all Genotypes at 200 IU/mL except the one sample that was not quantifiable. This sample (Genotype 2a/2c) was detected using the Multiprep Specimen Processing Procedure, but was negative when tested using the Standard Specimen Processing Procedure. This result

is consistent with HCV RNA levels below the detection limit of the assay. Data are provided in Table 8.

Table 8: HCV Genotype Samples Tested

HCV Genotype/Subtype	Quantity	Reactive Total (Multiprep)	Reactive Total (Standard Prep)
1	8	8/8	8/8
1a	3	3/3	3/3
1b	9	9/9	9/9
2	1	1/1	1/1
2a	2	2/2	2/2
2b	10	10/10	10/10
2a/2c	3	3/3	2/3*
3a	12	12/12	12/12
3a	6	6/6	6/6
3e	1	1/1	1/1
4	1	1/1	1/1
4	11	11/11	11/11
4a	2	2/2	2/2
4c	3	3/3	3/3
4c/4d	2	2/2	2/2
4h	1	1/1	1/1
5a	2	2/2	2/2
6a	2	2/2	2/2

** One sample contained HCV RNA at a level below the Limit of Quantitation of a quantitative assay. Sample was tested undiluted.*

e. Sensitivity with Seroconversion Panels

Nine anti-HCV seroconversion panels were tested using both the Multiprep and the Standard Specimen Processing Procedures. Each specimen in each panel was tested by the Ortho HCV, version 3.0 ELISA Test system and all samples with reactive EIA results were also tested by Chiron RIBA HCV 3.0 SIA. The HCV RNA test results were then compared to the EIA test results for each specimen to determine if HCV RNA testing detected the presence of HCV infection prior to seroconversion.

The COBAS AmpliScreen HCV Test, v2.0 detected HCV infection an average of 32 days before seroconversion for the nine seroconversion panels. The data are summarized in Table 9.

Table 9: HCV Seroconversion Study

Panel	Day Positive Ortho 3.0 EIA and Chiron RIBA 3.0	Day Positive AmpliScreen v2.0	Difference AmpliScreen vs EIA
6212	14	0	14
6224	19	0	19
6215	20	0	20
9047	28	0	28
9045	41	0	41
6225	78	39	39
6213	43	11	32
6222	40	17	23
6227	74	0*	74*
Mean Days Earlier Detection			32

** Specimen was RNA positive on Day 0 but negative on Days 22 and 24. Day 74 specimen was RNA positive again*

3. Analytical Specificity

a. Analytical Specificity: Interfering Microorganism

The analytical specificity of the COBAS AmpliScreen HCV Test, v2.0 was evaluated by testing a panel of microorganisms and other disease states, including 23 viral isolates, two bacterial strains and one yeast isolate. No-cross reactivity was observed with the COBAS AmpliScreen HCV Test, v2.0. Table 10 summarizes the microorganisms studied.

Table 10: Analytical Specificity — Microorganisms Tested

Adenovirus type 2	Epstein Barr Virus	HIV-1 Subtype D
Adenovirus type 3	Hepatitis A Virus	HIV-2
Adenovirus type 7	Hepatitis B Virus (n=3)	HTLV-I
Autoimmune samples	Herpes Simplex type 1	HTLV-II
Candida albicans	Herpes Simplex type 2	Human Herpes Virus 6
Chlamydia trachomatis	HIV-1 Subtype A	Human Herpes Virus 7
Coxsackievirus B1	HIV-1 Subtype B	*Staphylococcus epidermidis*
Cytomegalovirus	HIV-1 Subtype C	Varicella-Zoster
Echovirus 1		

Up to ten individual patient plasma specimens from each of the following disease categories were spiked with low levels of HCV-positive plasma (within 2-3X the 95% LOD): HIV-1, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans.* No false negative test results were observed.

b. Analytical Specificity: Specificity with Non-HCV Hepatitis Samples

Twenty-five HAV- and 25 HBV-positive specimens (all HCV-negative) were tested for cross reactivity with the COBAS AmpliScreen HCV Test, v2.0 by using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be negative. No false positive test results were observed.

These samples were also spiked with low levels of HCV-positive plasma and tested using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be positive. No false negative test results were observed.

4. Effect of Interfering Substances

a. Endogenous Interfering Substances

HCV-spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of bilirubin (up to 20 mg/mL), triglycerides (up to 3000 mg/dL), hemoglobin (up to 1.0 g/dL), and albumin (up to 6 g/dL) were tested.

These endogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HCV Test, v2.0, using either the Standard or Multiprep Specimen Processing Procedure.

b. Exogenous Interfering Substances

HCV-spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of aspirin (up to 50 mg/mL), pseudoephedrine-HCl (up to 3 mg/dL), ascorbic acid (up to 20 mg/dL), acetaminophen (up to 40 mg/dL), or ibuprofen (up to 40 mg/dL) were tested. These exogenous substances did not interfere with the sensitivity or specificity using either the Standard or Multiprep Specimen Processing Procedure.

5. Uracil-N-Glycosylase (UNG) Performance

AmpErase (uracil-N-glycosylase, UNG) catalyzes the degradation of DNA containing deoxyuridine, but not DNA containing thymidine or RNA containing uridine. Deoxyuridine is not a constituent of the HCV Target RNA, but is always present in amplicon. In the AmpliScreen HCV Master Mix reagent, version 2.0 deoxyuridine triphosphate replaces thymidine triphosphate as one of the dNTPs. Only target amplicon contain deoxyuridine is susceptible to UNG-mediated degradation prior to amplification of the target DNA. Therefore, AmpErase is an effective countermeasure against inadvertent amplicon carryover.

6. Reproducibility Studies

The reproducibility of the Test was established by testing two six-member EDTA plasma panels with known concentrations of HCV. Panel One was tested using the Multiprep Specimen Processing Procedure contained one HCV-negative sample and HCV-positive samples with HCV RNA concentrations of 10, 25, 50, and 50,000 IU/mL. Panel Two was tested using the Standard Specimen Processing Procedure contained one HCV-negative sample and HCV-positive samples with concentrations of 25, 50, 100 and 50,000 IU/mL.

Testing was performed at three sites with two operators at each site using three COBAS AmpliScreen HCV Test, v2.0 kit lots. Each operator used a dedicated COBAS AMPLICOR Analyzer throughout the study. Each operator was provided panel sets that had been randomized and labeled in blinded fashion.

All valid reproducibility data were evaluated by calculating the percentage of correct results for each panel member. The data were analyzed by site, lot, testing day, run, and operator for each Specimen Processing Procedure (Multiprep and Standard).

The reproducibility study for the COBAS AmpliScreen HCV Test, version 2.0 demonstrated consistency by lot and site for both the Multiprep and Standard Specimen Processing Procedures as seen in Table 11 and 12 below:

Table 11: Reproducibility Results— Multiprep Specimen Processing Procedure

Results By Lot (Number Positive/Number Tested)					
	Negative	10 IU/mL	25 IU/mL	50 IU/mL	50,000 IU/mL
Lot #1	0/89	72/89	164/177	88/90	90/90
(%)	(0%)	(81%)	(93%)	(98%)	(100%)
Lot #2	0/90	59/90	168/180	88/89	90/90
(%)	(0%)	(66%)	(93%)	(99%)	(100%)
Lot #3	0/90	59/90	170/179	88/89	90/90
(%)	(0%)	(66%)	(95%)	(99%)	(100%)
Results By Site (Number Positive/Number Tested)					
Site #1	0/90	66/89	166/178	88/89	90/90
(%)	(0%)	(74%)	(93%)	(99%)	(100%)
Site #2	0/89	65/90	170/179	90/90	90/90
(%)	(0%)	(72%)	(95%)	(100%)	(100%)
Site #3	0/90	59/90	166/179	86/89	90/90
(%)	(0%)	(66%)	(93%)	(97%)	(100%)

Table 12: Reproducibility Results— Standard Specimen Processing Procedure

Results By Lot (Number Positive/Number Tested)					
	Negative	25 IU/mL	50 IU/mL	100 IU/mL	50,000 IU/mL
Lot #1	0/90	56/89	166/180	89/90	90/90
(%)	(0%)	(63%)	(92%)	(99%)	(100%)
Lot #2	0/90	66/89	165/179	89/90	90/90
(%)	(0%)	(74%)	(92%)	(99%)	(100%)
Lot #3	3/87	68/90	167/179	89/90	90/90
(%)	(3%)	(76%)	(93%)	(99%)	(100%)
Results By Site (Number Positive/Number Tested)					
Site #1	0/87	61/89	162/179	85/87	90/90
(%)	(0%)	(69%)	(91%)	(98%)	(100%)
Site #2	1/90	72/90	169/179	88/90	90/90
(%)	(1%)	(80%)	(94%)	(98%)	(100%)
Site #3	2/90	57/89	167/180	88/90	90/90
(%)	(2%)	(64%)	(93%)	(98%)	(100%)

B. Clinical Trials Summary

1. Pool Reactivity in Volunteer Blood Donors

A random selection of 8,240 pools revealed that 117 Primary Pools were reactive for an initial reactive rate of 1.42%. There were 106/117 (90.6%) positive pools that were concordant with confirmed positive serology status. None of these pools were identified as having a window period case. A total of 11 pools were found positive but were not confirmed positive by serology or by subsequent testing of individual donations by the COBAS AmpliScreen Test, v2.0. Results are summarized in Table 13.

Table 13: Pool Reactivity in Volunteer Blood Donors

Category	Pools	Percentage
Pools Tested	8,240	100
Non-Reactive Pools	8,123	98.58
Initially reactive pools	117	1.42
Initial pools with concordant serology	106	1.28
Positive pools due to window case	0	0
Initial Pools with negative serology and negative individual donation AmpliScreen Testing (false positive)	11	0.13

A random selection of approximately 250,000 specimens was selected from geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS AmpliScreen HCV Test, v2.0. Using the antibody results, the HCV status of each specimen was determined. HCV status-negative included either: 1) anti-HCV EIA negative, regardless of other results (unless the subject was enrolled in the follow-up study and had test results that changed this assessment); or 2) anti-HCV EIA positive and RIBA negative.

HCV status-positive included either: 1) anti-HCV EIA repeat reactive and RIBA positive; or 2) anti-HCV EIA repeat reactive or HCV RNA positive upon follow-up. HCV status-unknown included anti-HCV EIA repeat reactive with RIBA indeterminate or unknown.

There were 247,998 specimens that were determined to be HCV status-negative. Of these, 247,990 were also HCV RNA-negative. The specificity of the COBAS AmpliScreen HCV Test, v2.0 in this study was 247,990/247,998 or 99.997% with 95% confidence limits of 99.99% to 100.00%. The negative predictive value obtained by summing all the cases determined to have HCV status negative among the 247,990 COBAS HCV tested donations is estimated in this study to be 99.95% with exact 95% confidence limits (99.94% , 99.96%).

There were 243 specimens that were determined to be status-positive. Of these, 203 were also HCV RNA-positive. The positive predictive value obtained by finding the percentage of specimens detected to be HCV status positive among 203 COBAS positive donations is estimated to be 94.42% with exact 95% confidence limits (90.45%, 97.08%). All 243 samples in this population were included in the analysis, irrespective of HCV RNA titers. These data are consistent with previous reports that about 20% of HCV seropositive samples will have undetectable HCV RNA.

2. Single Donation Testing Performance

A total of 2,515 blood donor specimens were tested individually in the COBAS AmpliScreen HCV Test, v2.0 clinical trial. Of the 2515 specimens, five were classified as HCV seropositive and were removed from the calculation of specificity. Of the 2,510 specimens tested, 2,508 were HCV RNA negative and two were HCV RNA positive. No follow-up was conducted on these two donors and they were presumed to be false positive. The specificity of the COBAS AmpliScreen HCV Test, v2.0 in this study was 99.92% (2,508/2,510) with a 95% confidence interval of 99.71% to 99.99%.

3. Sensitivity in a Chronic HCV Population

Fifty-eight specimens were obtained from patients with a diagnosis of chronic HCV disease. All specimens were confirmed to be serologically positive by a licensed anti-HCV EIA followed by RIBA 3.0. The specimens were tested undiluted using the Standard Specimen Processing procedure and diluted 1:24 using the Multiprep Specimen

Processing procedure. All specimens were positive in the COBAS AmpliScreen HCV Test, v2.0 by both specimen processing procedures.

4. Detection of Window Period Cases

From April 8, 1999 to December 31, 2000, approximately 7 million donations were tested. During this period there were 20 confirmed window period cases detected. A confirmed window period case is defined as an enrolled individual from whom the index donation was positive with the COBAS AmpliScreen HCV Test, v2.0 but non-reactive by EIA for anti-HCV, and a follow-up specimen was shown to be anti-HCV EIA repeat reactive using the Abbott HCV EIA 2.0 assay and/or the Ortho HCV Version 3.0 ELISA test system and/or HCV RNA positive. The detection rate of such window period cases was 0.00029% (1 in 350,000) with a 95% confidence interval of 0.00017% to 0.00041%. In addition, four subjects with negative serology and no follow-up specimens were presumed to be window period cases, as a specimen from the plasma bag for each confirmed the index HCV RNA positive result. If these four subjects are included, the detection rate of window period cases is 0.00034% (1 in 292,000) with a 95% confidence interval of 0.00021% to 0.00049%.

5. Sensitivity in a High Risk Population

Specimens were prospectively collected from a patient population being evaluated at hematology clinics for biochemical, clinical and/or histological evidence of liver disease and/or evidence of HCV infection. Specimens were tested in a blinded fashion with COBAS AmpliScreen HCV Test, v2.0 using the Standard Specimen Processing Procedure.

Fifty-seven of 62 total specimens were positive for HCV RNA. Four specimens negative for HCV RNA were also negative for HCV antibody by both a licensed screening EIA and confirmatory assay and were excluded from the analysis. The COBAS AmpliScreen HCV Test, v2.0 detected 57 out of 58 HCV antibody-positive specimens.

V. PACKAGE INSERT

A copy of the Package Insert (directions for use) is attached.